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STUDY OF THE EFFECTS OF URANIUM ON KIDNEY FUNCTION

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A Report prepared by Dr. Michael A. Moss, Dalhousie University, Halifax, under contract to the Atomic Energy Control Board.

ABSTRACT

The chemical toxicity of inhaled or ingested soluble uranium, especially its nephrotoxicity surpasses its radiotoxicity. Because of the functional overcapacity of the kidney, it is probable that adverse effects on renal function due to occupational uranium exposure may be sustained long before this becomes evident. The advent of more sensitive and specific tests, particularly of proximal tubular dysfunction, suggests that it may now be possible to detect and monitor such sub-clinical effects on renal function. The Atomic Energy Control Board requires an updated review of uranium nephrotoxicity, and an evaluation of the various tests available which might be used to detect altered kidney function in uranium workers. Recommendations are also required regarding occupational studies which could be conducted in these workers to assess their current state of kidney function and possibly serve as monitoring tools in the future.

RÉSUMÉ

Les caractéristiques chimiques toxiques, surtout néphrotoxiques, de l'uranium soluble respiré ou avalé sont plus dangereuses que ses propriétés radiotoxiques. À cause de la surcapacité fonctionnelle des reins, il est probable que des effets nuisibles sur la fonction rénale, dus à l'exposition professionnelle à l'uranium au travail, puissent être subis bien avant qu'ils soient cliniquement évidents. L'introduction de tests plus sensibles et plus précis, particulièrement du dysfonctionnement des tubes proximaux, laisse supposer qu'il pourrait maintenant être possible de déceler et de contrôler de tels effets infracliniques sur la fonction rénale. La Commission de contrôle de l'énergie atomique a besoin d'une mise à jour des caractéristiques néphrotoxiques de l'uranium, ainsi que d'une évaluation des divers tests disponibles qui pourraient être utilisés pour déceler des défaillances rénales chez les travailleurs de l'uranium. Elle a également besoin de recomandations concernant des études qu'il serait souhaitable de conduire sur ces travailleurs, afin d'évaluer leur fonction rénale actuelle et de servir éventuellement de moyens de contrôle dans l'avenir.

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EXECUTIVE SUMMARY

The major objectives of this study were to:

- provide an updated literature review of the effects of uranium on kidney function
- assess the significance of exposure to the various compounds present in the nuclear fuel cycle
- evaluate candidate markers of uranium nephrotoxicity, for potential use in occupational monitoring
- develop recommendations for further studies required, and
- to develop a contact list of researchers active in this area

A comprehensive literature search covering the years 1978 to 1988 was conducted using the Medline and Embase (Excerpta Medica) data bases. Relevant publications were retrieved and reviewed. Site visits to two nuclear fuel production facilities were arranged, and this also provided the author with useful background information regarding the nuclear fuel production process, and current occupational health monitoring policies and procedures. During the final stages of the study, a contact list of researchers in this field was prepared. Contacts were successfully established with a number of individuals who are still active in this area, and also with some workers in related areas of interest, such as renal tubular function following exposure to other metals, such as cadmium.

Uranium is a classic nephrotoxin, and its use in high dosage for the experimental induction of nephrotoxicity has been well established. The hexavalent (uranyl) ion predominates in vivo, and this has a high affinity for sulphydryl, phosphoryl and carboxyl groups. A uranyl bicarbonate complex is the prevalent diffusible form circulating in the blood. Uranium rapidly distributes from the vascular compartment, primarily to bone and kidney; the renal component being largely excreted in the urine. Instability of the uranyl bicarbonate complex in the renal tubular urinary filtrate results in the release of uranyl ion and potential for binding with tubular cell proteins and interaction with high energy phosphate molecules. Renal tubular dysfunction appears to be the earliest manifestation of adverse health effects due to uranium. Experimental exposures in animals resulting in renal tissue uranium concentrations in the range 1-3 μg/g lead to renal tubular changes which are rapidly followed by adaptive and regenerative changes. These findings have been reproduced with various uranium compounds in experimental models. It seems unlikely that renal tissue uranium levels in the occupationally-exposed would approach these levels, but confirmatory

data is lacking in this area. There is no evidence to suggest that the health effects of some forms of uranium found in the nuclear fuel production cycle are insignificant, but there is a clear theoretical basis for the assumption that the more insoluble compounds may be of lesser significance. However, the significance for low level uranium exposure in the occupational setting is uncertain, since occupationally-induced chronic renal disease has not been reported in this context.

The advent of very sensitive indices of renal tubular function provide the opportunity to screen for subclinical functional alterations in the occupational setting. There are two main categories of these markers: urinary enzymes and low molecular weight proteins. A number of these are critically reviewed in this report, and a panel of tubular markers is recommended for use in pilot studies.

In a final section of the report, an assessment is made of the more significant information gaps which remain in this field. Recommendations are made for a pilot occupational study of the renal tubular function of uranium workers, using workers from a non-nuclear facility as a control population. The goal of such a study would be to validate the use of markers of renal tubular function in the routine occupational health monitoring of uranium workers.

This report also contains recommendations for the development of a biochemical investigational protocol to be used following any accidental acute uranium exposure, and also for consideration to be given to the establishment of a Canadian uranium registry.

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1. UPDATED REVIEW OF URANIUM NEPHROTOXICITY

Task 1 Update the review of the effects of uranium on kidney tissues given by PE Morrow in "Biokinetics and Toxicology of Uranium" (Proceedings of the Colloquium: Biokinetics and Analysis of Uranium in Man. Richland, WA, Aug. 1984).

Task 2 Update the sections of the Stopps and Todd report (AECB Info-0074) dealing with kidney damage caused by uranium uptake.

These two tasks cover the same territory and will be addressed in one section. A comprehensive literature search was conducted over a retrospective 10 year period, primarily through the Medline data base and Embase (Excerpta Medica). In the interest of continuity, this update summarises some previous literature referenced in the previous reports noted above and integrates this with more recently published material.

1.1 Metabolism and urinary excretion of uranium

The metabolism of uranium has been reviewed[1,2]. It is a highly toxic element when administered parenterally as soluble salts, and is much less toxic when ingested, due to a low coefficient of uptake from the gastrointestinal tract. Recent studies of the absorption and biokinetics of uranium in rats have been reported[3]. Gastrointestinal absorption was 0.6-2.8% over the oral gavage dosage range studied (0.003-45 mg U per kg body weight). The relative distribution of the uranium body burden between kidneys and skeleton changed with time. The kidney:skeleton ratio of the retained dosage was 1.5 at 4 hours, but only 0.2 at 10 days.

In vivo, uranium is present in tetravalent or hexavalent (uranyl) forms, the latter being more stable. Tetravalent uranium introduced into the bloodstream either oxidises to the hexavalent species or a colloidal form in the plasma. The uranyl cation forms reversible complexes with sulphydryl, phosphoryl, and carboxyl groups of cell membranes. Uranyl compounds will combine readily with proteins and nucleotides, forming stable complexes[4,5]. In the vascular compartment, a non-diffusible uranyl-albumin complex is formed in equilibrium with a diffusible ionic uranyl bicarbonate complex (UO_2HCO^{3+}). The uranyl bicarbonate complex is stable, but will dissociate under conditions of reduced pH, such as in bone, and in the kidney tubule[6]. The uranyl ion replaces calcium in the hydroxy-apatite complex of bone crystals[5]. Hexavalent uranium is rapidly excreted by the kidney; the excretory rate is partly dependent upon the pH of the tubular urine[5]. At high pH, most of the uranyl complex is stable and is excreted in the urine. If the pH is low, the complex dissociates to a variable degree, and the uranyl ion may then interact with cellular proteins

in the tubular wall. This results in reduced uranyl excretion, and the protein-binding can impair tubular function. There is also evidence from dog studies that the uranyl ion competes with ATP for a binding site on the Ca^{2+} and Mg^{2+} adenosine triphosphatase (ATPase), and that this may be a factor in its renal toxicity[7]. Biphasic urinary excretion has been observed following an acute single dose. First there is a rapid phase during which 70% of the absorbed dose is excreted during the first 24 hours, followed by a much slower phase with a half-life of many months[5].

1.2 Acute uranium nephrotoxicity

1.2.1 Pathobiological course of uranyl nitrate-induced nephrotoxicity

Uranium salts are among the earliest examples of substances used experimentally to induce mammalian renal failure, and uranyl nitrate is the most common salt used for nephrotoxic modelling[8]. The spectrum of acute uranium nephrotoxicity has been studied extensively in animals, and to some extent in man, and includes morphological changes, enzymuria, glycosuria, increased excretion of amino acids and small proteins, and at higher doses, albuminuria and acute renal failure[9-16]. Comparative nephrotoxicity studies must be interpreted with particular attention to species, route of administration, formulation of uranium, and frequency and duration of exposure. Of the common laboratory animals, the rat appears to correspond most closely to humans in sensitivity to intravenously administered uranium.

The necrosis seen after uranyl nitrate administration begins in the proximal pars recta (S_3 region) at the corticomedullary junction and progresses both proximally and distally from that site. Hyaline casts (accumulations of proteinaceous debris), or casts containing shredded necrotic cells, are present at all levels of the tubular system. In an acute-exposure rat model, there is apical cellular swelling, vacuolisation and loss of the brush border microvilli of the cortical proximal tubule. By the fifth day of exposure, frank necrosis of the S_2 and S_3 segments is evident[17]. In a longer term study, the S_2 and S_3 segments of the pars recta were the most consistently damaged portion of the nephron. In these rats, follow-up 8 weeks after challenge demonstrated marked interstitial fibrosis with a mononuclear cell infiltration, suggesting chronic injury[18].

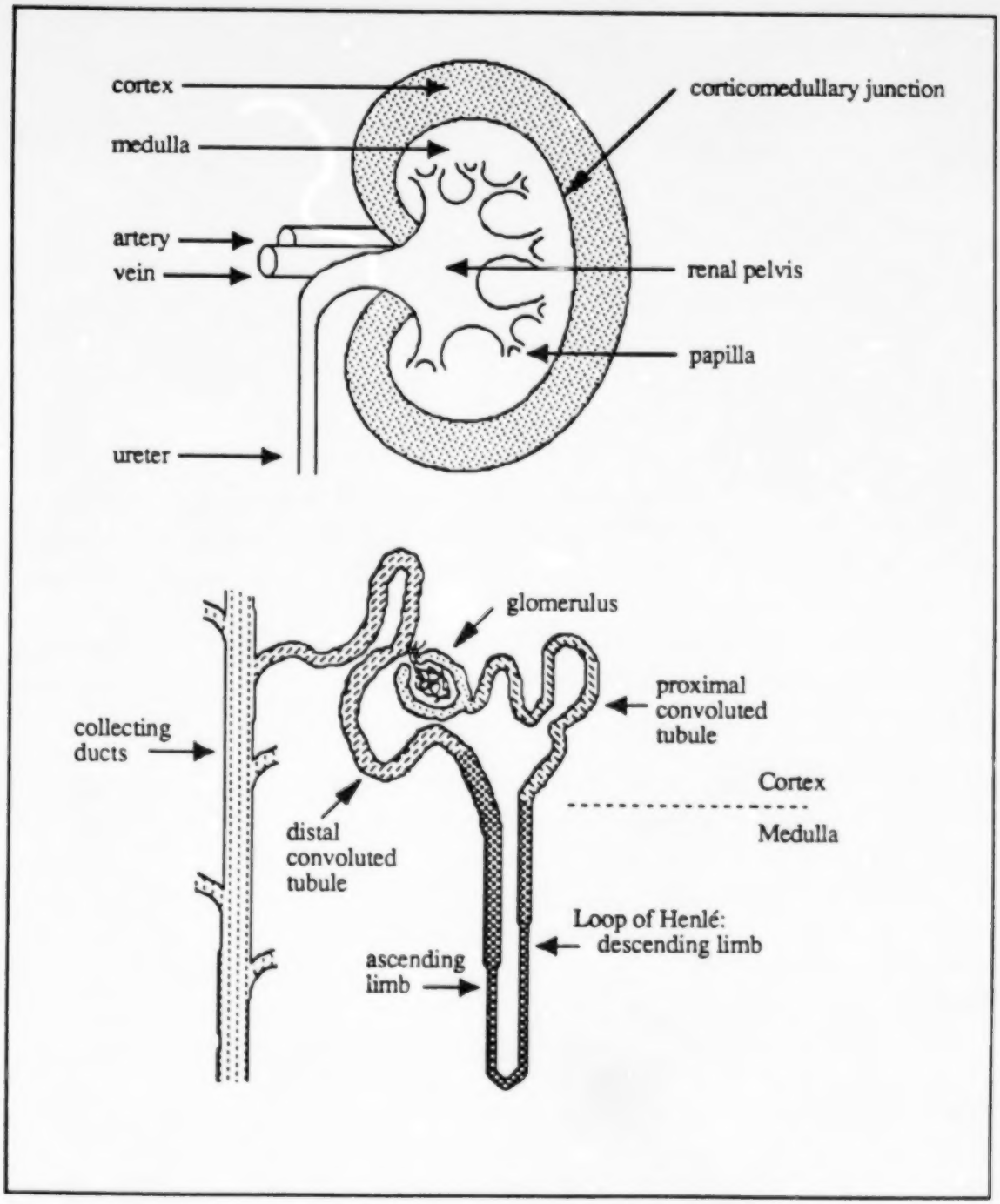


Figure 1 **Structure of the human kidney and a representative nephron**

Some other aspects of uranyl nitrate-induced polyuric acute tubular necrosis have been recently reported in the male Sprague Dawley rat[19]. Uranyl nitrate (UN) was given intravenously, as a single dose of 5mg/kg, 15mg/kg or 30 mg/kg body weight. These doses induced weight loss, polydipsia (excessive thirst) and polyuria (passage of large volumes of urine) within 24 hours compared to the controls which were treated with 5% sodium bicarbonate. Twenty four hours after the injection of UN, serum creatinine and blood urea nitrogen levels had increased. These changes continued for at least 72 hours. Light microscopic studies conducted 24 hours after injection revealed partial degeneration and necrosis of the proximal tubules and many casts in the distal convoluted tubules. These changes progressed for 72 hours. Despite this tubular damage, the glomeruli were relatively intact. Five days after injection, the epithelial cells lining the proximal tubules displayed regenerative activities; these findings were more prominent after 10 days. Destruction of mitochondria in the proximal tubular cells was revealed by electron microscopy. Mitochondrial disruption leads to an inhibition of oxidative phosphorylation, and a decreased supply of adenosine triphosphate (ATP) required for the reabsorption of sodium and water. This is presumed to account for polyuria and polydipsia observed at this stage in the acute dose model. Ten days post injection, regenerative activities in the proximal tubular cells showed concomitant maturation of intracellular organelles. The regenerated epithelium described in such studies differs from normal in that it has relatively short cells with a markedly decreased number of organelles, vestiges of short microvilli, and round to oval-shaped mitochondria[19].

It is of interest that diabetes in the rat confers partial protection against the nephrotoxicity of UN[20]. It has been suggested that this may partly be due to decreased binding and transport of the nephrotoxin into proximal tubules.

The typical findings outlined above in acute nephrotoxicity have been reproduced in several other animal models, including the mouse[21], chick[8], and dog[22].

1.2.2 Observations in uranyl fluoride-induced nephrotoxicity

Uranyl fluoride (UO_2F_2) also produces an acute nephropathy. It primarily causes damage to the pars recta of the proximal tubule, particularly around the corticomedullary junction[23-25], the same site vulnerable to the nephrotoxic effects of other uranium-containing compounds, such as UN[17,26]. At present, there is no explanation as to why these tubules in particular are principally affected by uranium compounds[27].

Following UO_2F_2 exposure, the urinary excretion of several enzymes including lactate dehydrogenase (LD) and aspartate aminotransferase (AST) has been shown to increase in several

studies[23,24,27]. This increase is delayed until after the second day, which suggests that if urinary markers are being used to assess exposure effects, then the monitoring process should be carried out for at least 1 week after the suspected exposure[27]. Although it is difficult to extrapolate from the rat, there is some evidence to suggest that the urinary enzymes gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), alanine aminopeptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG) may be less sensitive than LDH and AST in assessing the nephrotoxicity of UO_2F_2 [24,27]. The reversibility of renal injury in the rat following UO_2F_2 exposure has been described[25]. Two exposure groups of rats were administered uranyl fluoride by intraperitoneal injection over a period of 24 days. The cumulative doses were 0.66 and 1.32 mg U/kg body weight. This achieved peak levels of uranium in kidney at or above 3.4 or 5.6 μg U/g (wet weight) respectively. A renal injury characteristic of uranium-induced nephropathy was produced, which was followed by a rapid repair phase and complete recovery within 35 days of the last injection. Following a careful histological analysis, it was concluded that histological changes occurred in rats having renal burdens in the 0.7-1.3 μg U/g range. Much more marked changes were associated with renal tissue uranium levels in the 1.3-3.5 μg/g range[25].

The nephrotoxicity of UO_2F_2 has been studied in unilaterally nephrectomised rats, in order to determine whether this would be enhanced following a reduction in renal mass[27]. It was found that the nephropathy was not enhanced by unilateral nephrectomy. This suggests that following UO_2F_2 exposure, individuals with 50% reduced renal mass (due to surgery, age or disease) are probably not at significantly greater risk of nephrotoxicity than their colleagues.

1.2.3 Glomerular alterations in acute uranium nephrotoxicity

Glomerular alterations have been described in acute renal failure induced by intravenous uranyl acetate in the rabbit[28]. Fifteen hours after injection (2 mg/kg body weight), the glomerular diameter was reduced, and creatinine clearance decreased to 55% of controls. A flattening and spreading of podocyte cell bodies associated with loss of epithelial foot processes was observed, with a reduction in the density of endothelial fenestrae. Glomerular filtration decreased to approximately zero after 5 days, the time at which tubular damage was most pronounced. Glomerular alterations reverted to normal within 14 days, with good recovery of glomerular function. Similar studies with uranyl nitrate in a rat model have shown that the decrease in glomerular filtration coefficient is potentially preventable and reversible, using a combination of treatments with converting enzyme inhibitor and plasma volume expansion[29]. It has been reported elsewhere that the beneficial effect of saline loading in uranium-induced acute renal failure is associated with lessened cast formation rather than with suppressed renin-angiotensin activity or enhanced urinary uranium excretion[30]. The onset of acute renal failure following UN

administration in the rat has been reported to be associated with increased glomerular biosynthesis of the eicosanoids (vasodilator hormones) prostaglandin E_2 and prostacyclin[31]. It was suggested that this may play a significant role in the homeostatic regulation of renal perfusion and glomerular filtration after acute toxic injury to the kidney.

1.3 Exogenous factors influencing uranium nephrotoxicity

Two recent publications are of relevance when comparing published data on experimental nephrotoxicity. It has been recently shown that acclimation to caging has a significant effect on the nephrotoxic response of experimental rats to uranium. This is apparently related to a lower water consumption by naive (non-acclimated) animals and results in a lower tolerance to uranium[32]. Elsewhere, studies in the rat have shown that dietary protein intake prior to as well as following UN administration can have a significant effect on subsequent renal function and survival[33]. Those animals on restricted protein diets exhibited improved survival. It was suggested that this might be due to a smaller glomerular filtration load being delivered to the tubules or reduced tubular metabolic activity.

1.4 Critical dose for uranium nephrotoxicity

Rats receiving parenteral ^{nat}U- and ^{235}U-enriched UN showed marked renal morphological changes at a dose of 50 µg/kg (with an estimated renal dose of 10 µg/g)[34]. Literature regarding human acute exposures is sparse. Uranium workers exposed accidentally to high concentrations of airborne uranium (as U_3O_8, UF_6, or uranium ore concentrate) have reportedly shown no apparent renal injury although urinary uranium concentrations exceeded 1 mg/L[35]. Elsewhere, four human volunteers were given an oral dose of 10 mg uranium (corresponding to an average dose of 0.12 mg U/kg as uranyl nitrate hexahydrate). They reported no subjective symptoms and did not demonstrate proteinuria[36]. The threshold tissue concentration for renal injury in Man remains uncertain, but is likely to be above 0.6 µg/g, and may be as high as 3 µg/g[37]. For metabolic modelling purposes, a conservative estimate of 1 µg U/g renal tissue has been proposed[37]; this value also approximates to the 0.7-1.3 µg/g range observed in the rat model described above[25].

2. HEALTH HAZARDS FROM URANIUM COMPOUNDS PRESENT IN THE NUCLEAR FUEL CYCLE

Task 4. Using currently available information, indicate whether it can be demonstrated that the health hazards from uranium in the form of compounds that are present in the nuclear fuel cycle, are low enough to be neglected.

2.1 Uranium burden of Reference Man

Natural uranium concentrations in soft tissues and bone of New York City residents (autopsy cases) have recently been reported[38]. The bone concentration was found to be a factor of 10 lower than previously reported for this tissue, and a revised uranium burden of 8 μg was estimated in comparison with the ICRP Reference Man estimate of 67 μg[39]. The authors suggest that if this data is accepted, then strict reliance on Reference Man values could invalidate conclusions based upon bioassay measurements of present or former workers with the uranium industry[38]. This may be significant in the evaluation of the relative hazards of the various forms of uranium present in the nuclear fuel cycle.

2.2 The nuclear fuel cycle

Workers are exposed to uranium in mining and processing of the material, recovering trace amounts of uranium from phosphate mining, and in the generation of nuclear power[40]. Many different chemical forms of uranium can be encountered during the nuclear fuel cycle. Experience has shown that inhalation is the most common mode of occupational exposure, although uranium can be absorbed through the skin, through wounds or ingested[21]. The more soluble compounds are generally regarded as having greater chemical toxicity. Yellowcake is chemically complex, but contains 26-86% ammonium and magnesium diuranate, which is regarded as biologically soluble[9]. Yellowcake is refined by nitric acid digestion to an intermediate product, uranyl nitrate, which is thermally decomposed into uranium trioxide (UO_3). This in turn is either converted to uranium dioxide (UO_2) as an end product, or reacted with hydrofluoric acid to form uranium tetrafluoride (UF_4), which is calcined and further fluorinated to produce uranium hexafluoride (UF_6). There are therefore two products of the nuclear fuel cycle: UO_2 and UF_6. Uranium dioxide is pressed and sintered into high-density fuel pellets, inserted into zircalloy tubes assembled in bundles, and serves as fuel in heavy-water reactors. Uranium hexafluoride undergoes enrichment for ultimate use in light-water reactors.

2.3 Assessment of health hazards of uranium compounds in the nuclear fuel cycle

2.3.1 Yellowcake and uranyl nitrate

Because of their solubility, the various forms of uranium present in yellowcake must be considered as potentially hazardous. The toxicity of UN has been well documented, since it is the form of uranium most commonly used in experimental nephrotoxicity.

2.3.2 Uranium trioxide

Uranium trioxide presents a potential inhalation hazard to nuclear fuel workers. The mobilisation of uranium trioxide after its deposition in the lung in rats, dogs, rabbits, cats and man has been reviewed[41]. Metabolism of UO_3 dust after deposition in rat lung has been studied[41]. It was shown that UO_3 delivered by inhalation is rapidly cleared from the lungs, mainly to the blood. The subsequent distribution of uranium among body tissues, and the fraction of the systemic content excreted in urine, was similar to that obtained for other transportable hexavalent uranium compounds. The authors concluded that urine monitoring data would be of more value than lung radioactivity counting measurements for assessing occupational human exposure, and that for setting exposure limits by inhalation, UO_3 should be considered a highly transportable compound. This implies that intakes by workers should be restricted to those recommended for short-term exposures and not those based on an annual limit.

2.3.3 Uranium dioxide

Uranium dioxide (UO_2) also presents a potential inhalation hazard to workers. Although uranium dioxide is one of the most insoluble uranium compounds, it has been shown in the rat that provided the skin barrier is avoided (eg subcutaneous implantation), uranium dioxide freely penetrates the animal's tissues and promptly affects target sites such as kidney and bone[42]. Toxicological effects were characterised by acute renal failure, with progressive lesions in the epithelium of cortical proximal convoluted tubules, similar to those reported in a uranyl nitrate model[17]. The authors concluded that caution in handling this compound should be recommended, because minute amounts penetrating through small skin wounds represent a serious risk for those engaged in daily manipulation of uranium, especially those related with the nuclear fuel industry[42].

The metabolism of ceramic and non-ceramic forms of UO_2 have been studied in the rat[43]. It was concluded that the lung retention and translocation kinetics of uranium are not affected appreciably by the different methods of production of the dioxide, and that both materials should be assigned to

inhalation class Y as defined by the International Commission on Radiological Protection. Most of the uranium that was translocated to the blood was rapidly excreted in the urine. The principal sites of uranium deposition in extrapulmonary tissues were the kidneys and skeleton. The distribution of uranium amongst body tissues and the relationship between systemic content and cumulative urinary excretion indicated that it was transported in the hexavalent form, probably as a carbonate complex. The authors recommended that exposure limits to UO_2 should be based on radiation dose rather than chemical toxicity.

2.3.4 Fluorides of uranium

Uranyl fluoride (UO_2F_2) is the principal hydrolysis product of uranium hexafluoride (UF_6), which is used for the industrial production of enriched ^{235}U for nuclear fuel and weapons. Since the UO_2F_2 is produced from the spontaneous hydrolysis of UF_6 in room air, there is a potential for significant exposure to workers when UF_6 is accidentally released into room air or the environment[27,44]. Recently reported studies suggest that uranium tetrafluoride (UF_4) may be more highly transportable after deposition in the lungs than was previously thought[45].

2.3.5 Metabolism of high-specific-activity uranium isotopes

Little information exists on the metabolism and potential health effects of ^{233}U and ^{232}U, high-specific-activity uranium isotopes associated with thorium breeder reactors. The distribution and retention of these isotopes following inhalation of UN aerosols or intratracheal installation has been described in the rat[46]. Uranium in this form was translocated rapidly from the lung and was retained mainly in skeleton, kidney and liver. Clearance from kidney was rapid, equivalent to one-quarter to one-half of the initial lung burden during the first day after inhalation.

2.4 Occupational studies

By comparison with acute toxicity studies in animals, relatively little is known about the chronic effects of occupational exposure in man, which has been reviewed by Thun et al[9]. It is not clear what minimum level or duration of exposure is needed to produce toxicity in humans, and human data are so scant as to make it difficult to draw conclusions[40]. In a study commissioned by the Manhattan Project in the late 1940s, increased excretion of the tubular enzyme catalase was found in the urine of 46 chemical workers exposed to uranium[47]. In a later study, increased aminoaciduria was shown in workers exposed to uranium hexafluoride[48]. In a more recent study, kidney function was evaluated among 39 uranium mill workers and a control group of 36 local cement plant workers (matched for age, sex and race)[9]. Assays for urinary uranium showed that during 1975-1978, 115 of 535 (21%) measurements of uranium in urine from the mill workers had

exceeded the upper action level of 30 µg/l. Both dust exposures and the concentrations of uranium in the urine declined in mid-1979, when a new mill facility was completed. The study was conducted approximately 2 years later. The uranium workers showed a significantly higher urinary excretion of β_2-microglobulin (β_2-m) and five amino acids than the reference group. A dose-effect relationship was shown between the clearance of β_2-m, relative to that of creatinine, and the length of time that the uranium workers had spent in the yellowcake drying and packaging area, the work area with the highest exposures to soluble uranium. Detailed employment records were not available, but questionnaire data was obtained on work history, medical history and exposure to certain extraneous nephrotoxins. The excretion rate of β_2-m was significantly higher among the uranium workers when expressed in a variety of ways, including clearance and clearance relative to creatinine, although the urinary concentration of β_2-m was itself not significantly different between the groups, nor did the urinary β_2-m concentration of any worker exceed the upper reference limit cited by the test manufacturer (370 µg/l). Serum β_2-m levels were significantly higher among the uranium workers, 23% having levels at or above the given upper reference limit of 2.4 µg/ml for males under 50 years of age. This elevation was not explicable on the basis of reduced glomerular function, since their serum creatinine and creatinine clearance were equivalent to their age-matched referents. The elevated serum β_2-m is consistent with increased production, a phenomenon which is well recognised in the context of cadmium exposure. The excess aminoaciduria among the uranium workers was characterised by an increased excretion of dicarboxylic amino acids and methionine (a neutral amino acid). It may be significant to note that none of the uranium workers had been exposed to air concentrations of soluble uranium above the existing US occupational standard of 1×10^{-10} µCi/ml (3.7 Bq/m^3) for two years prior to the study, although air levels had frequently exceeded that level before 1979. This study could therefore have underestimated reversible renal effects. Only 3 of the workers had worked full time in the yellowcake area for more than 10 years, therefore these exposures were of relatively short duration. The severe renal tubular disease seen in workers exposed to heavy metals such as cadmium usually appears following prolonged exposure of 20 years or more. The authors draw attention to the lack of epidemiological studies to evaluate renal tubular function in uranium workers, and the fact that routine monitoring has not been conducted using sensitive measures of tubular function, since the semi-quantitative measurement of urinary albumin is an insensitive test for tubular injury.

2.5 Retrospective cohort mortality studies

A retrospective cohort study of over 2000 workers employed in a uranium mill for at least one year before 1972 has been reported[49]. Mortality from most causes was lower than expected. In particular, stroke and cardiovascular disease were only 80% of expected. This was attributed to the

"healthy worker" effect seen in many working groups. Significant excess risks were found only for nonmalignant respiratory disease (ONMRD) and miscellaneous accidents, but not for pulmonary or lymphatic malignancies. The excess mortality due to nonmalignant respiratory disease was due to a category which includes emphysema, fibrosis, silicosis, and chronic obstructive respiratory disease. Mortality due to this subcategory was 2.5 times that expected. From the available data, it was not possible to distinguish the contributions to risk of ONMRD from uranium mill exposures to silica, vanadium pentoxide, uranium ore dust, and yellowcake, or even from prior non-uranium mining exposures. The work histories of the cases of ONMRD did not cluster in any particular area of the uranium mills. Nonsignificant excesses were found for lymphatic malignancies after 20 years latency and for death due to chronic nephritis among short term workers. Six individuals died with chronic renal disease (versus 3.6 expected). Three of these deaths were from chronic glomerulonephritis; the 3 other deaths occurred in older individuals, and either prostatic obstruction or carcinoma was also noted on the death certificate. It was concluded that these latter 3 deaths were from secondary renal disease not associated with uranium exposure. Uranium mill employment was brief for all these cases, and induction time varied from 4-19 years. The excess deaths due to chronic renal disease could therefore not be clearly attributed to occupational exposure.

Another retrospective cohort mortality study of 995 uranium processing workers has also been reported[50]. These workers had been employed at a plant in Buffalo, New York, which was in operation from 1943 to 1949. Belgian Congo pitchblende and domestic uranium ores were converted in this facility to uranium tetrafluoride. Intermediate products in the uranium processing operations were uranium oxide, dioxide, trioxide, and hexafluoride. The study was restricted to those white males who had worked at the plant for more than 30 days (females and non-white males were excluded because they were few in number). Two comparison groups were used, the white male population of the United States and the white male population of the New York counties of Erie and Niagara. Significantly increased standardised mortality ratio values were obtained for: all causes, laryngeal cancer, atherosclerotic heart disease (in contrast to the study above) and pneumonia. The observed number of lymphatic and lung cancers were essentially as expected. Exposure monitoring data was incomplete, but no association was found with length of employment or work in the most hazardous areas of the plant.

In summary, it would appear from the information above that the potential for adverse health effects from uranium compounds present in the nuclear fuel cycle cannot be dismissed, although serious health effects have not been recognised in the few retrospective studies reported. There

remains a need to better define actual versus potential health effects in the occupationally-exposed subject, and an assessment of the clinical significance of such effects if detected.

3.0 EVALUATION OF CANDIDATE MARKERS OF URANIUM NEPHROTOXICITY

Task 5. Indicate the various tests that are available to detect sub-clinical, clinical, temporary or permanent kidney damage; discuss their merits and weaknesses in general (including sensitivity, ease of use, cost, etc), and in particular the extent to which they are able to detect damage due to uranium uptake.

3.1 Commonly used tests of renal function

The kidneys have a large functional reserve capacity, and clinical renal function tests may be very insensitive in the early detection of nephrotoxicity[51]. Commonly used serum parameters of "renal function" such as creatinine and urea concentration, only become abnormal when a significant impairment of organ function has been sustained. Serum urea and creatinine are used as indices of glomerular filtration rate (GFR). Dietary protein intake can have significant effects upon the serum urea concentration. Both serum urea and creatinine are inferior to the more cumbersome estimation of creatinine clearance, which also involves the collection of a timed (eg 24 hour) urine sample. In the early stages of glomerular disease, both serum urea and creatinine concentrations show only a small change in absolute terms[52]. For example, a halving of GFR will result in a doubling of serum creatinine, but for some individuals, this serum creatinine may still fall in the reference range. GFR has to be less than 30% of the age and sex corresponding normal mean before serum creatinine becomes higher than the upper reference level with certainty[53]. This low sensitivity is thought to be caused mainly by an increasing fractional urinary excretion of creatinine via tubular secretion when GFR declines from normal to moderately decreased levels. There is wide interindividual variation in normal serum creatinine concentrations, and longitudinal studies (serial sampling on the same individual) may be more informative than a single value compared with normal subjects in the population[54]. These tests are therefore insensitive in the early stages of renal dysfunction. In order to detect sub-clinical renal lesions at an early stage, much more sensitive parameters of function or injury are required. The determination of ribonuclease, β_2-m and lysozyme in serum have been assessed for the detection of reduced GFR within the normal range of creatinine. Ribonuclease and β_2-m showed similar diagnostic efficiency, the former being recommended by the authors because of its cheaper and simpler analysis[55].

3.2 Selection of tests to screen for occupational nephrotoxicity

Due to the multiple functions of the kidney, no single test or group of tests can be relied upon to detect all classes of nephrotoxicity. Screening tests and specific tests to measure glomerular or tubular function must be selected to match the pattern of nephrotoxicity[56].

Tests available for the evaluation of occupationally induced renal disease have been discussed in two excellent papers by Thun and Clarkson (1986)[54], and Lauwerys and Bernard (1987)[57]. It is now recognised that the kidney is a critical organ for some industrial exposures, such as to cadmium and uranium, and there is increased interest in occupational monitoring of parameters of renal function or injury[58]. There is a wide gap between the invasive and sophisticated tests used to measure renal function in the hospital setting and the crude tests available in the workplace. The traditional occupational screening program usually measures only serum urea and creatinine, and simple semiquantitative tests for the presence of blood or protein in urine. There are some instances where more specialised approaches have been employed, for example the measurement of urinary β_2-microglobulin excretion in cadmium-exposed workers. Most renal insults are categorised as to whether they involve primarily the glomerulus, the proximal tubule, the distal tubule, the interstitium or the renal vasculature. This classification is simplistic, because many toxic substances affect more than one tissue, depending upon dose, age of the exposed person and the chronicity of exposure. For many types of renal injury, by the time the patient is symptomatic, the end result is non-specific renal disease, for which the original aetiology and pathology may be unrecognisable[59]. However, since the purpose of occupational screening is to identify pathology before the advent of end-stage renal disease, the site of the primary renal lesion can guide the selection of an appropriate screening strategy.

3.3 Screening tests for proximal tubular injury

3.3.1 Proteinuria vs enzymuria

In the case of uranium, the primary area of renal involvement is the proximal tubule[54]. Proximal tubular damage due to various insults is probably very common, but regeneration is rapid, and mild injury will not be clinically apparent unless special tests are employed. Besides uranium, many compounds can cause proximal tubular necrosis, including other metals, salicylate, sulphonamides, radiographic contrast media and antibiotics such as cephaloridine and the aminoglycosides[60]. Most publications in this area are in the context of agents other than uranium.

In routine clinical practice, renal tubular function or damage is rarely specifically tested. It is relatively difficult to detect, and the generalised loss of tubular function in chronic renal diseases accompanies the loss of glomerular function and in most settings does not need to be assessed separately[61]. In recent years there has been a rapidly increasing interest in the detection of renal tubular damage and in the assessment of renal tubular function. Simpler techniques have become available to measure the urine components that form the basis of these sensitive tests of tubular function, and these approaches will be reviewed here.

There are two general categories of tests of proximal tubular dysfunction[54]. One group measures increased urinary excretion of those substances that are normally reabsorbed from the urinary filtrate by the proximal tubules. The second group measures components arising from cells in the kidney[62]. Following tubular injury, the concentration of amino acids and small molecular weight proteins (< 40,000 daltons) in the urine may be greatly increased, due to decreased tubular reabsorption. Substances that have been measured as markers of this process include the amino acids, β_2-microglobulin (β_2-m), and retinol-binding protein (RBP)[54]. Quantitative measurement of urinary amino acids is well established but expensive, and may be insensitive in chronic or interstital renal disease[63]. Radioimmunoassay for small proteins such as β_2-m has been more commonly used[54].

In an effort to identify new diagnostic features of tubulointerstitial nephritis (TIN), urinary total protein concentrations and urinary excretion rates of 4 serum proteins and of proximal tubular antigens were determined for 5 normal subjects and for 44 patients with various well-characterised renal diseases[64]. Four urinary protein indices were found to correlate with TIN:

clearance of lysozyme/clearance of albumin > 20	($C_{LZM}/C_{alb} > 20$)
clearance of immunoglobulin G/clearance of albumin % > 55%	($C_{IgG}/C_{alb}\% > 55\%$)
renal tubular epithelial antigen (RTE) excretion > 10 units/min	($U_{RTE} > 10$ U/min)
urinary albumin/urinary total protein concentrations < 50%	($U_{alb}/U_{TP}\% < 50\%$)

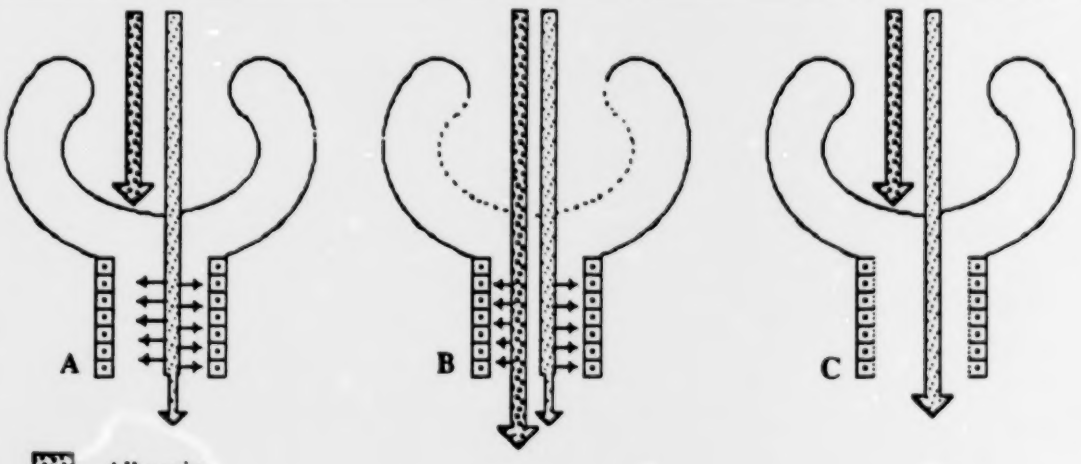
Among 21 TIN patients, 18 were positive to 1 or more of these criteria, whereas 17 of 19 patients with glomerulonephritis (GN) were negative to all four. It was determined that even if only 2 of the criteria were used ($U_{alb}/U_{TP}\% < 50\%$; $C_{LZM}/C_{alb} > 20$), a positive result for either would suggest a TIN proteinura, whereas a negative result on both would imply a glomerular process. These criteria would have correctly discriminated 88% of the patient population studied[64]. This type of multi-phasic approach deserves further evaluation.

The alternative investigational approaches of proteinuria and enzymuria outlined above will now be discussed in more detail.

3.3.2 Proteinuria

(a) Tubular proteinuria It is generally accepted that the upper limit for urinary protein excretion in healthy adults is in the region of 150 mg/day in the absence of exercise or febrile illness[52,65]. Injury to the glomerulus typically increases glomerular permeability, permitting the

passage of high molecular weight proteins the size of albumin (69,000 daltons) or larger to pass into the urinary filtrate. On the other hand, injury to the renal proximal tubules prevents the usual reabsorption of low molecular weight proteins from the filtrate, permitting these to appear in increased concentrations in the urine[54,62,66,67]. This phenomenon is known as tubular proteinuria[68,69], and has been recently reviewed[70]. Low molecular weight proteins are largely taken up by pinocytosis in the proximal renal tubules and then degraded to the amino acid level in the lysosomes of the tubular cells. The reabsorption of large proteins, such as albumin, is unselective and occurs in the distal parts of the proximal tubules. The more proximal reabsorption of small proteins is possibly selective and seems to be a more vulnerable process[70]. An increased urinary excretion of low molecular weight proteins can be due to an increased plasma concentration with overload of the reabsorptive capacity rather than to tubular damage. It is therefore strictly necessary to measure the plasma concentrations of the protein, particularly β_2-m, the production of which is increased in a variety of diseases[61].



(A) Normal: albumin is largely restricted at the normal glomerulus; low molecular weight proteins are freely filtered, and a great proportion reabsorbed and catabolised by the normal proximal tubule.

(B) Glomerular defect: increased permeability to albumin results in albuminuria; renal handling of low molecular weight proteins is unaltered. Proteinuria may be gross and is non-selective.

(C) Tubular defect: proximal tubular defect reduces uptake of low molecular weight proteins, which are therefore excreted in increased amounts in the urine. Renal handling of albumin is not affected.

Figure 2 Schematic diagram of tubular proteinuria

As a further cautionary note, because the renal uptake of proteins involves a saturable process, the urinary excretion of low molecular weight proteins such as RBP and β_2-m specifically reflects the reabsorptive capacity of proximal tubules only when the glomerular filtration rate is normal or only slightly impaired (ie serum creatinine <20 mg/l = 177 µmol/l)[71].

Abnormalities in the urinary excretion of RBP or β_2-m are frequently observed in the absence of a decrease of GFR and even of a detectable proteinuria. In many situations (eg trauma, short-term antibiotic therapy), these changes are probably not clinically relevant and represent no more than a transient subclinical deterioration in tubular function[71]. However, in the instance of long-term occupational exposure to a nephrotoxin, an increase in the urinary excretion of low molecular weight proteins might indicate a progressive loss of tubules, which because of the large reserve capacity of the kidney, would remain clinically silent until a considerable amount of kidney parenchyma was irreversibly damaged[72]. Published experience with RBP is rather more limited than that with β_2-microglobulin (β_2-m), which has been employed for several years as a monitoring tool in occupational cadmium exposure[73].

(b) Analytical considerations for the determination of urinary protein Analytical approaches for the quantitation of proteinuria have been compared[54]. The most commonly used screening tests for urine protein are the dipstick methods containing bromphenol blue. These tests have a detection limit for free albumin of 20-30 mg/100ml, but are insensitive to globulins and may miss lower levels of proteinuria in dilute urine[52]. A much better semiquantitative test for protein is the precipitation of protein by heat and trichloroacetic acid. This test has a lower detection limit of 5-10 mg/100 ml and is more sensitive to globulins. In order to detect early or subclinical increases in protein excretion it is preferable to use an analytical test which yields quantitative results. Otherwise, the imprecision and insensitivity of the laboratory results will limit the interpretability of the findings[54]. The sulfosalicylic acid turbidity method is a good quantitative assay for total protein determination, and can be recommended for this application. Radioimmunoassay (RIA) procedures are now also available for albumin and other specific proteins; the RIA method for albumin is capable of quantifying very low levels and this detection of "microalbuminuria" is currently being widely evaluated in the context of early or potential diabetic nephropathy. Although relatively expensive, this approach is probably the state of the art for sensitivity in albumin quantitation at this time.

With modern analytical techniques, it is now possible to resolve at least 250 proteins in urine[54]. One-dimensional electrophoresis on sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) achieves separation of urinary proteins on the basis of molecular mass. This has been used to demonstrate cadmium-induced tubular proteinuria[74]. Two-dimensional electrophoresis allows greater resolution on the basis of separation by charge as well as molecular mass. This technique is

still considered a research tool and requires further clinical validation, but it does provide more detailed fractionation of urine proteins than is available by other means[54]. Tubular proteinuria in a worker exposed to thorium has been demonstrated by this technique[62].

3.3.3 Enzymuria

(a) Origins of urinary enzymes Enzymes released into the urine comprise a second major category of markers of renal tubular injury[54,62,75]. Enzymuria has received less attention as a marker of proximal tubular injury than has low-molecular weight proteinuria, and its utility in occupational studies remains unestablished[54]. Renal tubular cells are rich in many enzymes and these may be released into the urine following cell damage and necrosis, thus providing a sensitive index of nephrotoxicity[60]. At least 40 enzymes have been recognised as potential markers for the diagnosis of urorenal diseases[76]. There is little published information on normal values for these analytes in controlled population groups[77,78]. Histochemical studies have not shown significant segmentation in the distribution of enzymes within the proximal and distal parts of the nephron[79], and it would appear that urinary enzyme analysis does not have high specificity for the site of a particular lesion. Urinary enzymes which have received the most attention include alkaline phosphatase (ALP) and gamma glutamyltranspeptidase (GGT) present in the brush border of the proximal tubular cells, aspartate aminotransferase (AST) and lactate dehydrogenase (LD) in the cytosol, and N-acetyl-β-D-glucosaminidase (NAG) in the lysosomes. Urinary enzymes may also originate from other sites, reducing their diagnostic specificity. An international symposium was held in 1978 on the biochemical aspects of the diagnostic value of urine; the proceedings have been published and address many of the issues arising from the diagnostic applications of urinary enzymes and proteins[80].

(b) Use of enzyme panels Urinary enzymes may reflect both extrarenal and renal disease states, and a higher degree of specificity might be achieved by use of a panel of enzymes rather than one particular parameter. Following evaluation of 10 enzymes (AAP, arylsulfatase A, ALP, transamidinase, β-glucuronidase, GGT, alpha-glucosidase, NAG, lysozyme and LD), the use of a panel of 3 enzymes has been recommended[81] for clinical use:

i) a typical brush border enzyme, eg alanine aminopeptidase (AAP)

ii) a lysosomal enzyme, eg β-glucuronidase

iii) a low molecular weight protein which passes through the glomerular membrane and which is usually reabsorbed by the tubuli, eg lysozyme

Elsewhere, among a panel of enzymes evaluated, the determination of lactate dehydrogenase (LD) and gamma glutamyltranspeptidase (GGT) were found to be the most sensitive markers of early renal tubular damage, based on a rat model exposed to mercuric chloride[82]. Because of difficulties in interpretation of the results obtained, these authors cautioned that such urinary enzyme determinations be reserved for use in specialist centres.

(c) Variations in urinary enzyme excretion Diurnal and sex-related variations in urinary enzyme excretion have been described[83]. More recently, infradian (within-day) biorhythms of enzymuria have been reported for dipeptidyl peptidase IV, GGT and alanine aminopeptidase (AAP)[84]. These rhythms showed changes in the range of 100% in healthy individuals. It has been recommended that urines be collected for a 3-hour period (eg 6 a.m. to 9 a.m.) rather than over a 24 hour period, to minimise loss of enzyme activity through degradation, and also to standardise the procedure and minimise intra-individual variations in enzyme excretion[85]. Others have recommended that several urine samples of each subject be collected on consecutive days, and that the calculated mean or median value (after removing all outliers) be used as a basis for diagnosis[84]. This aspect of enzymuria appears cumbersome and imprecise. Urine enzyme activity also varies considerably according to the urine flow rate[76].

(d) Enzyme activity in urine: interfering factors Factors affecting enzyme activity in urine have been reviewed[86]. Several types of interference impair the assay of enzyme activity in urine[87]:

presence of various activators and inhibitors (endogenous compounds, drugs, metabolites)
sensitivity of enzymes to the wide ranges of urinary pH
low stability of enzymes in dilute solutions
possible interference of urochromes (urinary pigments) with spectrophotometric assays
spurious changes in enzyme activity have also been observed

Inhibitors need to be removed or diluted out, otherwise falsely low values of enzyme activity may be obtained. The presence of an inhibitor can easily be detected by plotting the activity against the volume of urine used in the assay. Normally, a straight line plot would be expected, but if inhibitors are present a curve is obtained. The inhibitors usually have a low molecular weight and can be removed by dialysis. This procedure is time-consuming and may be unsuitable for handling a large number of samples. An alternative to dialysis is to dilute the urine, so that the effect of the inhibitors becomes negligible. This is the most convenient method, provided a sensitive assay procedure is employed. Various other methods of urine preparation for enzyme assays are available, such as centrifugation, dilution, gel filtration[87], ultrafiltration[88], as well as less

common techniques such as ion exchange chromatography, salting-out and freeze drying. Gel filtration is particularly suitable for the removal of low molecular mass interferences.

Besides the presence of inhibitors, the usefulness of urinary enzymes is limited by the causes of increased excretion other than tubular cell damage (eg drug therapy such as aminoglycosides), and because the increased secretion may not be sustained in spite of a continuing insult[61].

(e) Stability of enzymes in the urine sample Long term storage of the sample leads to a progressive decline in enzyme activity, with a significant loss of activity after only one day in some cases. Deep freeze storage (-18°C) should be avoided, since freezing and thawing of enzymes incurs even greater losses. Ideally, samples should be stored at 4 °C after collection and assayed on the same day[86]. Four urinary treatment methods have been evaluated for the stabilisation of AAP, GGT and NAG in normal urines[89]. Among dialysis or the addition of albumin, dimethyl sulfoxide, or glycerol, it was found that glycerol treatment (2.5 ml of a 10% V/V solution of glycerol added to a 22.5 ml aliquot of urine) was the most reliable method for stabilising these enzymes whilst frozen.

(f) Urinary enzymes - other analytical considerations Although spectrophotometric methods have often been applied to the determination of urinary enzymes such as aryl sulphatase A (ASA), β-galactosidase (GAL)[90], N-acetyl-β-D-glucosaminidase (NAG)[90], β-glucuronidase (GRS), LD[91], GGT, ALP and leucine arylamidase (LAS), they tend to be limited by high imprecision in the low activity range. Less attention has been paid to these determinants in external laboratory control programs, and recommendations have been made regarding the preparation of a pooled urine control which could be used in an internal quality assurance program[87].

(g) Serum enzymes reflecting renal injury? Enzymes that normally do not appear in the serum but can be found in the kidney include transamidinase, trehalase and guanase. Increased activity of these enzymes in serum may occur following renal injury, and although published material in this area is sparse, it is an area of increasing interest.

3.4 Monitoring renal injury - the cadmium experience

3.4.1 Cadmium nephrotoxicity

There is a wealth of published data on experimental, occupational and environmental cadmium nephrotoxicity. In the assessment of potential markers of proximal tubular injury or dysfunction in the context of uranium exposure, a brief review of the relevant cadmium literature is valuable. Most such data relates to the use of specific proteins such as β_2-m and RBP. The effects of cadmium on

the kidney have been recently reviewed[92]. In long term exposure to cadmium, the kidney is considered the critical organ, the earliest detectable abnormality being the onset of tubular proteinuria. The measurement of low molecular weight proteins in the urine (eg β_2-m) is now routinely performed to assess the integrity of proximal tubular function in workers exposed to cadmium. This cadmium-induced proteinuria seems to progress slowly, but once started it is usually irreversible. Because of the lack of consistent progression to renal insufficiency, some authors consider that an isolated increased β_2-microglobulinuria has no health significance, and this is an issue of some controversy. Follow up studies are required to assess the predictive value of increased urinary excretion of such specific proteins in workers exposed to cadmium[92].

3.4.2 Renal tubular markers in occupational cadmium exposure

The effect of 10 years' cadmium exposure was investigated among a total of 18 workers (including 5 control workers)[93]. Indicators of previous exposure (blood and urinary Cd and β_2-m) were compared with clinical estimates of renal tubular function (tubular reabsorption of phosphate and β_2-m). Elevated blood Cd was significantly correlated with elevated serum β_2-m. The study demonstrated that increased β_2-m in urine is not necessarily due to tubular impairment, but may also be the result of the increased β_2-m in plasma exceeding the renal threshold. It is known that serum β_2-m may be elevated in chronic Cd exposure, but the mechanism is uncertain.

Elsewhere, renal function was assessed (urinary β_2-m, RBP and albumin) in a group of 53 cadmium solderers[94]. The workers were stratified into multiples of 5-years exposure. Renal tubular dysfunction was found in 17 of the 31 subjects with more than 5 years exposure, and in one this was also associated with glomerular dysfunction. Comparison of the mean β_2-m and RBP values for each exposure period showed that both proteins remained within normal limits up to 15 years exposure, then increased considerably. These proteins appeared to have comparable sensitivity in detection of tubular proteinuria in this study. Liver and kidney burdens of cadmium were assessed in 25 of the solderers by in vivo neutron activation analysis; 22 had raised liver and kidney cadmium concentrations, and included some managerial staff who had not been actively involved in soldering for several years. This emphasises the need to monitor all personnel with potential exposure to nephrotoxins, even if their duties do not involve direct exposure to the agent in question. Urinary cadmium concentration did not increase in parallel with the onset of low molecular weight proteinuria. It is thought that tubular proteinuria occurs at a lower kidney cadmium concentration than that at which the kidney fails to retain stored cadmium, when there is a pronounced increase in urinary cadmium concentration. Since the urinary excretion of uranium is pH-dependent[5], it is also unreliable as an index of renal injury; a low urinary uranium does not necessarily infer that tubular dysfunction is not present.

Cadmium-induced renal effects were examined by another group in 60 workers previously exposed to cadmium[95]. Tubular damage in the form of β_2-microglobulinuria was found in 40%. There was also increased albumin excretion; it was suggested that this was secondary to the tubular damage since in no case was typical glomerular proteinuria found that could be related to cadmium. In a subsequent publication, the same authors further reported that the prevalence of β_2-microglobulinuria was strongly related to the cumulative cadmium dose estimate[96]. There was no significant relationship between the concentration of β_2-m in serum and urine, indicating that the β_2-microglobulinuria was due to tubular damage and not secondary to an increased endogenous production. Repeated examinations of 19 workers between 1976 and 1983 revealed that in almost all cases the β_2-microglobulinuria was irreversible[96].

3.4.3 Other renal markers in cadmium exposure

Circulating anti-glomerular basement membrane (GBM) antibodies have been identified in workers exposed to cadmium[57]. Anti-laminin antibodies were increased when the urinary excretion of cadmium exceeded 20 µg/g creatinine. This finding was not present in a similar study of lead workers. The diagnostic significance of such anti-GBM antibodies deserves further evaluation.

Decreases of erythrocyte and glomerular membrane negative charges have been demonstrated in chronic cadmium poisoning of rats; a similar decrease in erythrocyte negative surface charge was shown in cadmium-exposed workers who presented no or only subclinical signs of renal injury[97]. This suggests that RBC charge determination might have some predictive value for the development of proteinuria under certain conditions such as chronic cadmium poisoning. The analytical technique used was based upon the binding of a cationic dye (alcian-blue 8GX) to erythrocytes or tissue homogenates in vitro.

3.5 Specific candidate markers of proximal tubular injury or dysfunction

3.5.1 Enzymes

(a) N-acetyl-β-D-glucosaminidase (NAG) NAG is a lysosomal enzyme with a molecular weight of 130-140,000[60] and is not detectable in serum. NAG has been shown to be a sensitive indicator of renal injury. It is the enzyme which has been most commonly measured as an index of renal tubular injury[61] and has proven to be one of the most valuable[57]. Among a series of renal transplant patients, increased NAG in urine was evident in 70% of cases of acute rejection 1-3 days before any other test parameter was positive[98]. However, analysis was recommended within 45 minutes of collection. It has been noted that NAG is unstable in alkaline urine (pH >8)[99,100].

Determination of urinary NAG has been evaluated as an indicator of renal tubular damage in persons with chronic cadmium poisoning[101]. It was found that urinary NAG increased with urinary β_2-m up to about 100 Units/g creatinine; above this value the NAG activity did not increase with further increases of β_2-m excretion. This was attributed to severe tubular cellular destruction in the more advanced stages of cadmium-nephrotoxicity, whereby the cells could not excrete any more enzyme into the urine. Elsewhere, mercury vapour-exposed workers in a thermometer factory were evaluated for renal effects using several parameters including NAG[102]. Significant correlations were found between urinary mercury levels and NAG activity, and NAG appeared to be a sensitive indicator of renal injury in this setting. Fluorometric assays have been described for the determination of NAG[103-105]. A positive interference has been noted using a fluorometric assay in two individuals taking Dyazide[89]. It is unclear whether this was due to an in-vivo effect or to analytical interference. A spectrophotometric assay has been described[106] using an *m*-cresolsulfonphthaleinyl substrate (MCP). This has been adapted to the Cobas Bio centrifugal analyser[107], and the Hitachi 705 analyser[100].

(b) Alanine aminopeptidase (AAP) AAP is a brush border enzyme with a molecular weight of 157,000[60]. Its excretion in urine has been reported to be a sensitive index of osmotic nephrosis, chronic pyelonephritis, and adequacy of therapy for hyperthyriodism[81]. The enzyme is only stable in the untreated urine sample for 3-4 days at 4 °C; freezing destroys the activity instantly. It has been shown that storage stability is improved by addition of ethylene glycol to the sample[108]. An automated spectrophotometric assay has been described for the determination of urinary AAP[108].

(c) Lysozyme Lysozyme is an enzyme that catalyses the hydrolysis of bacterial cell walls. It occurs in high concentrations in many organs, including the kidney, as well as in tears, serum, neutrophilic granulocytes and cells of the mononuclear phagocytic system. Only small quantities are normally found in urine because of a very efficient tubular transport mechanism. Lysozyme is freely filtered at the glomerulus and reabsorbed almost exclusively in the proximal renal tubule. Elevated urinary lysozyme activity is found during urinary tract infections, proximal renal tubular damage, renal allograft rejection and excessive synthesis, whereby the reabsorptive capacity is overwhelmed. Increased urinary lysozyme activity therefore lacks specificy for tubular injury, although it is very sensitive in this regard. Lysozyme excretion is augmented in subjects undergoing a modest diuresis[109]. The mechanism for this remains unclear, but may relate to a slight decrease in tubular reabsorption which might occur with diuresis. It has been recommended that urinary lysozyme excretion be expressed as a fractional excretion (per mol of creatinine)[109]. This recommendation has also been made for a number of other urinary enzymes[110]. The renal

processing of lysozyme and release of lysosomal enzymes has been studied[111]. It has been shown that increased rates of exocytosis of lysosomal enzymes can be acutely induced without inducing demonstrable cellular injury. Classic analytical methods for the determination of lysozyme were based upon a bacteriolytic assay, but a method employing high pressure liquid chromatography (HPLC) has recently been described[112]. Samples require prepurification before chromatography.

(d) Lactate dehydrogenase (LD) LD is a ubiquitous cytosolic enzyme. Isoenzyme fractionation of LD has been reported to be useful in the differential diagnosis of renal and bladder infections, where the LD-5 isoenzyme fraction was shown to be increased in 97% of childhood renal infections, but not in cases of cystitis[113]. LD has also been shown to be a very sensitive indicator of experimental renal tubular toxicity[114]. Although it is very sensitive in this regard, it lacks specificity.

(e) Gamma-glutamyltranspeptidase (GGT) GGT is a brush border enzyme which catalyses the first step of glutathione catabolism. Urinary GGT has been used as a sensitive indicator of renal proximal tubular damage in experimental nephrotoxicity, but its interpretation is hindered by large intra- and interindividual variations[115].

(f) Trehalase Trehalase is an enzyme which hydrolyses trehalose to two glucose moieties. This enzyme is localised in brush borders of the intestine and of proximal renal tubules. Urinary trehalase has been evaluated as an early indicator of cadmium-induced renal tubular damage in the rabbit[116]. A significantly increased activity was found from 1 week after treatment, earlier than leucine aminopeptidase, alkaline phosphatase, proteinuria or glucosuria, without changes in the plasma trehalase activity. Elevated urinary trehalase activity has also been reported in inhabitants of the Jinzu river basin, an area which is known to be heavily cadmium-polluted[117]. In a further study, including subjects with Itai-Itai disease (a severe form of chronic cadmium posoning), urinary trehalase activity increased in the early stages, in parallel with urinary protein and β_2-m excretion[118]. At extremely high levels of urinary β_2-m, glucose or total protein, the urinary trehalase activity was rather lower. This was attributed to exhaustion of the enzyme consequent to the progressive proximal tubular damage.The analytical method for trehalase is susceptible to glucose interference, if present in the urine sample. Pretreatment of the urine is required by dialysis and concentration, before the substrate trehalose is added and glucose production measured.

3.5.2 Proteins

(a) β_2-microglobulin (β_2-m)

i) Source and renal handling of β_2-m β_2-m is the protein which has received the most attention as a marker of tubular proteinuria, and will be reviewed in detail here. The significance of β_2-m in the evaluation of renal function has been reviewed[70,119-123]. β_2-m has a molecular weight of approximately 12,000 and is present on the membrane of virtually every cell as the light chain of the Class I major histocompatibility complex (HLA antigens)[124,125]. As a result of the metabolism and degradation of HLA, β_2-m is dissociated from the heavy chain and appears in its free form in the extracellular fluid. About 95% of all free β_2-m is filtered by the normal glomerulus and subsequently almost completely reabsorbed and catabolised by the cells of the proximal tubules. The reabsorptive mechanism is a complex process which is still not clearly defined[126]. A normal kidney is able to reabsorb 99.9% of the filtered β_2-m, and maximally 370 µg/24 hr is excreted in the urine. Infusions of ornithine, arginine and lysine are known to cause transient tubular proteinuria, probably through temporary blocking of tubular membrane receptors. In the case of lysine, urinary β_2-m can rise to about 60% of the filtered load, until returning to normal within about one hour[127].

ii) Circadian rhythm of β_2-m excretion Urinary excretion of β_2-m has been reported to increase with advancing age above 50 years, in a process which may be unrelated to demonstrable tubular dysfunction[128]. A circadian rhythm of urinary β_2-m excretion was demonstrated in patients with nephrotic syndrome, which appeared different from the albumin rhythm[129]. Observations made in 10 healthy individuals also suggested the presence of circadian rhythm of β_2-m excretion in the absence of clinically important proteinuria. All individuals excreted more β_2-m in the urine during the day than during the night. It is unclear whether this phenomenon reflects a modulation in glomerular or tubular function, or both. It is likely that a similar situation exists for the excretion of other low molecular weight proteins, and this may be important in the interpretation of sequential data on such markers over short periods.

iii) Causes of increased urinary β_2-m excretion Many pathological conditions in which the proximal renal tubule is involved, are accompanied by an increased renal excretion of β_2-m as a result of reduced tubular reabsorption[60]. The clearance of β_2-m, expressed as a percentage of the clearance of albumin ($C_{\beta 2\text{-m}}/C_{alb}$), has been reported to a valuable marker in the assessment of tubulointerstitial nephritis[130]. Tubular recovery after relief of obstructive nephropathy in 21 patients has been studied through serial assessment of the urinary excretion of alpha-glucosidase, GGT, NAG and β_2-m[131]. Serum creatinine returned to normal within 9 to 26

days. Enzymuria returned to normal within 35 to 45 days, whereas normal urinary excretion of β_2-m occurred more than 100 days after relief of the obstruction. It was concluded that a prolonged period of impaired tubular function exists following restoration of normal glomerular function and ultrastructural recovery of the tubular cells; this may have implications for the assessment of renal tubular injury due to uranium exposure.

Increased urinary β_2m excretion has been observed in aminoglycoside nephrotoxicity and heavy metal exposure to cadmium[132] and mercury[133], among other disorders[119,134]. Increased urinary β_2-m excretion has also been reported in Balkan nephropathy, a chronic tubulointerstitial nephropathy of unknown etiology, with a high prevalence in some areas of Bulgaria, Romania and Yugoslavia[135]. Urinary β_2-m has been used as a marker of tubular dysfunction in pregnancy-induced hypertension, where it was shown that a tubular lesion occurs which is reversible following delivery[136]. It has also been used in the assessment of renal function of the transplanted kidney[137], in sick newborn infants[138], and as a marker of renal injury during treatment with the chemotherapeutic agent cis-platinum[139].

iv) Urinary β_2-m in uranium exposure Clinical studies from Nova Scotia reported on 324 persons exposed to variable amounts of uranium in their drinking water (up to 0.7 mg U/L) supplied from private wells[140,141]. There was no pattern of overt disease or symptomatic complaints which could be attributed to uranium exposure. Urinary β_2-microglobulin excretion (β_2m) was reported to show a trend effect with increasing uranium exposure, with evidence of reversibility for individuals who had stopped using the uranium-contaminated drinking water[140,141]. The excretion of urinary β_2-m among uranium workers has also been reported above[9].

v) Instability of β_2-m in acidic urine A major difficulty with the use of β_2-m as a screening test for tubular damage is its instability in acidic urine[142-144]. This instability may be due to enzymic degradation by one or more enzymes not active at an alkaline pH. β_2-m is unstable at room temperature in urine with a pH < 5.5. However, at body temperature, a rapid and irreversible loss of β_2-m occurs in urine with a pH < 6.0. As urine is normally stored in the human bladder for at least 2 hours before voiding, it has been advised to ensure production of urine with a pH ≥ 6.0[119]. This can be achieved by giving alkali to the subject before and during collection rather than adding alkali to the urine after voiding: 4 g of sodium bicarbonate on the evening preceding the collection, and another 4 g in four divided doses during the course of the 24 hour collection. To minimise degradation of spot samples, an alternative approach is to collect an aliquot of the sample in 5 ml tubes containing 0.5 ml of 10% sodium azide in a 0.07 M phosphate buffer solution (pH 6.5)[70]. This cannot ensure that degradation of β_2-m has not already occurred in the

bladder, however[144]. Elsewhere, a double-void technique has been employed, whereby the first morning void is discarded, and then a freshly voided sample is immediately alkalinised prior to freezing[145]. The foregoing is of considerable importance: in one study, the urinary pH of 80 workers was checked and 2 (2.5%) samples had a pH < 5.0 and 20 (25%) had a pH < 5.5[146]. Reliable estimates of urinary β_2-m therefore would require pre-collection subject preparation. Retinol binding protein (RBP) has been proposed as a preferable marker of similar molecular size to β_2-m, since it does not hydrolyze in the usual pH range of urine[147].

vi) Measurement of β_2-m Accurate radioimmunoassay methods are available for the measurement of β_2-m. Compared to radial immunodiffusion techniques, these are relatively expensive and the kits have a relatively short shelf-life because of the ^{125}I label used[148]. Radioisotope methods also have the disadvantages of high cost and prolonged assay time, in addition to requiring specialised laboratory facilities[149]. Where high volumes of samples must be processed, this may not be a disadvantage, and a semi-automated RIA procedure may be preferable. In addition, the RIA technique is more sensitive and accurate for a wider range of values[148]. A fast and simple competitive enzyme immunoassay has also been described for the determination of β_2-m in undiluted human serum, and has been used successfully in the analysis of urine samples[150]. An inexpensive micro-ELISA (enzyme-linked immunosorbent assay) technique has been described elsewhere, and is amenable to semi-automation[151].

(b) Retinol-binding protein (RBP)

i) Source and renal handling of RBP RBP has a molecular weight of 21,200 daltons, and plays a role in the transportation of retinol from the liver to the epithelial tissues[152,153]. In plasma, approximately 90% of RBP is bound to prealbumin. After retinol is delivered to target tissues, RBP loses its affinity for prealbumin and is rapidly eliminated from plasma by glomerular filtration and catabolism in the proximal renal tubules. The localisation of RBP in lysosomes in both the S_1 and S_2 segments of the tubules of the human kidney has been determined by immunocytochemical techniques[154]. This suggests that the reabsorption of RBP occurs in both segments.

ii) RBP vs β_2-m as a marker of tubular dysfunction The urinary excretion of RBP, normally about 100 µg/24 h, has been widely promoted as a reliable index of proximal tubular injury [155,156]. This parameter has been compared with β_2-m and NAG in patients with renal tubular damage due to a variety of causes (multiple injuries, rhabdomyolysis, antiobiotic treatment and poisoning by agents including solvents, heavy metals and pesticides)[71]. In almost all cases, RBP proved to be a more sensitive index of tubular damage than NAG, and preferable to β_2-m

because of its stability in acidic urine. These authors found that on the average, an increase in the urinary excretion of NAG becomes detectable when urinary RBP already exceeds the normal value by at least 10-fold. In urine with pH < 6.0, the RBP/β_2-m ratio increases as the pH decreases. This ratio can increase up to 500 in urines from patients with severe tubular injury as proteolytic enzymes are released from injured tubular cells and appear to degrade β_2-m much more than RBP[71]. The renal thresholds for the tubular reabsorption of free RBP and β_2-m are reached when the endogenous creatinine clearance is reduced by > 70% (serum creatinine ~ 177 µmol/l). From this stage of renal insufficiency, the urinary excretion of both proteins is no longer reliable for the assessment of renal function[156]. Urinary RBP and β_2-m excretion were compared in apparently healthy populations with and without occupational exposure to cadmium[157]. The relationship observed in neutral urine was: RBP (µg/mmol creatinine) = 0.786 + 0.814 β_2-m (µg/mmol creatinine). Weekly urine samples from the cadmium-exposed workers showed marked fluctuations not only in β_2-m, but also in RBP excretion. In some cases, the change was by almost one order of magnitude. The precise reasons for this were unclear, but this study indicates that even the pre-analytically more stable RBP is subject to intraindividual variation, and implies that repeated sampling and urine analyses are necessary to obtain more reliable data when using RBP as an occupational monitoring tool[157].

iii) Measurement of RBP A number of assays for the determination of RBP in urine have been reported using various analytical techniques such as radioimmunoassay[158,159], latex immunoassay[160], and enzyme-linked immunosorbent assay[161,162]. Radial immunodiffusion techniques are also available, but are of limited sensitivity when used with normal, unconcentrated urines[158].

(c) α_1-microglobulin (α_1-m) α_1-m has a molecular weight of 33,000, and has been assayed by radial immunodiffusion[163] and enzyme-linked immunosorbent assay (ELISA)[164]. It has been assessed as a marker of renal tubular dysfunction in cadmium exposure[165]. It appeared to be less sensitive than β_2-m in this regard, but is stable over a wider pH range (4.0-10.0). In another study using cadmium-poisoned subjects, urinary α_1-m was highly correlated with β_2-m under optimal pH conditions[146]. It was concluded that α_1-m seems to be more advantageous than β_2-m as an index of renal dysfunction because of its greater stability in urine. Combined measurements of α_1-m in the serum and urine have also been recommended in the estimation of glomerular filtration rate (GFR), and especially in the detection of a mild reduction in GFR[166].

(d) Adenosine deaminase binding protein (ADBP) ADBP is a proximal renal tubular cell antigen with a molecular weight of 120,000 daltons[167]. Both soluble and membrane forms of ADBP have been observed in the kidney. Since it is stable in alkaline urine, its measurement has

been advocated as a useful adjunct to some other markers of tubular injury or dysfunction such as NAG which are known to be unstable at pH >8.0[100,168]. ADBP has been shown to be stable in urine in the pH range 5.0-8.0, decreasing substantially at pH 4.0 as determined by sandwich enzyme-linked immunosorbent assay (ELISA)[169]. Low levels of ADBP have been detected in the plasma and urine of normal individuals, while significant elevations in urinary excretion have been shown in patients with various forms of renal injury. In one study, patients with glomerular disease were found to have higher than normal levels but could not be differentiated from patients with tubular damage in most cases[169]. It was concluded that this marker may have potential as a measurement of renal injury, but seemed non-specific. In a subsequent study, workers from the same centre reported improved diagnostic efficiency[170]. In this study, all 37 normal controls had urinary ADBP < 0.1 activity units (AU), with a mean value of 0.08 AU. Among 40 patients with glomerular disease, 29 had assay values < 0.2 AU, and 9 had values of 0.2-0.4 AU. Two others had values within the range 0.4-0.6 AU, but were both subsequently shown to have sigificant tubulointerstitial involvement in addition to their glomerular disease. In contrast to these results, among 79 patients with post-ischemic acute tubular necrosis, 51 had assay values > 1.0 AU, 23 had values of 0.6-1.0 AU, and 5 had values of 0.4-0.6 AU. From these data, the investigators concluded that this assay is a sensitive measure of renal proximal tubular injury and can reliably distinguish acute tubular from glomerular disease[170].

(e) Glycosaminoglycans (GAG) GAG are the major constituents of the anion layer of the glomerular basement membrane, which provides an electrostatic barrier to the filtration of anionic macromolecules such as albumin. The validity of urinary excretion of GAG as an index of the charge-selectivity of the glomerulus or as a non-specific marker of renal damage has been assessed[171]. Urinary excretion of GAG, albumin, RBP and NAG were determined in healthy subjects, diabetics, patients with acute tubular damage due to mutiple injuries, rabdomyolysis or acute poisoning, and in workers exposed to cadmium. In comparison with the control group, members of the study groups did show an increased prevalence of elevated urinary excretion of GAG. There was, however, no correlation within each group or in the total study population between the urinary excretion of GAG and that of albumin, RBP and NAG. It was concluded that the validity of urinary GAG as a marker of non-specific renal damage is questionable.

(f) Renal tubular epithelial antigen (RTE) RTE has also been proposed as a potential marker of tubular injury. It has been shown to have reasonable specificity as a marker of tubulointerstitial nephritis, but lacks sensitivity compared with brush border enzymes[130]. One theoretical advantage of measuring renal antigens in urine by specific immunological methods is the

fact that this approach avoids the interference of many factors (eg inhibitors, activators, stability) that may influence enzyme activity[57].

(g) Other candidate markers A reduced urinary kallikrein activity has been found in workers exposed to lead and cadmium[57]. Kallikrein is an enzyme which cleaves the decapeptide lysyl-bradykinin from a protein substrate and is synthesised by the kidney and released into the urine. Reduced activity of this enzyme could lead to a reduction in renal blood flow, and might be expected to occur in association with a decrease in prostaglandin E_2 (PGE_2). It is noteworthy to contrast this finding with the observation noted earlier that there is increased glomerular biosynthesis of PGE_2 in the rat following administration of uranyl nitrate[31]. The diagnostic utility of kallikrein and PGE_2 need to be more clearly defined in the assessment of nephrotoxins.

Two membrane proteins, maltase and gp330, present in the proximal tubule brush border, have been independently purified and found to be large glycoproteins of similar molecular weight (~300,000). They have been shown to be distributed differently in the brush border of the S_1 and S_2 segments of the proximal tubule. Maltase is concentrated on the microvilli, whereas gp330 is found in the apical invaginations located at the base of the microvilli[172]. Whether these proteins have a potential clinical diagnostic role as markers for different microdomains of the proximal tubular brush border is unknown at this time.

In addition to the above, the following have also been proposed as potential indicators of renal tubular dysfunction: alpha-glucosidase, malate dehydrogenase, aspartate aminotransferase, and urokinase[173]. These candidates have received less attention than those reviewed above and do not appear to offer any particular advantages.

3.6 Other aspects of urinalysis

3.6.1 Urinary concentration

The preferred method to assess urinary concentration is osmolality determination by freezing point depression. This may not be practicable in the field, and other approaches are commonly used. The use of specific gravity rather than osmolality is a source of imprecision[54]. Specific gravity shows good correlation with osmolality when the urine is dilute, but increasing divergence as the urine becomes more concentrated[52]. The use of a refractometer is preferable to specific gravity estimation, and is rapid and easy to use in the field.

3.6.2 Hematuria and microscopy

The presence of red blood cells and particularly, red cell casts in the urine sediment, is usually indicative of the presence of glomerular disease. Microscopic examination of the renal sediment is considered by many to be an indispensable part of the clinical examination of the kidney, yet neither qualitative nor quantitative microscopy has been used extensively in epidemiological studies of renal disease[54]. The lack of sensitivity of this test and the need to perform the analysis preferably within 2 hours of urine collection limits its application in occupational medicine[57]. For the valid performance of quantitative microscopy it is recommended[54] that the investigators must:

standardise the amount of urine studied
standardise either the centrifugation time, or use uncentrifuged urine
examine only fresh, clean voided samples to avoid degradation of casts in alkaline urine
have all samples read by a single examiner skilled in microscopy and cell counting

Small amounts of hemoglobin released by red cell lysis in hypotonic urine may also be detected by dipsticks impregnated with orthotoluidine. A positive test requires a microscopic examination to distinguish between hemoglobinuria and hematuria[57].

3.6.3 Measurement of uranium in urine

Measurement of uranium in urine has most frequently been performed using a sodium-fluoride fusion-fluorescence technique. A more sensitive method for determination of picogram quantities of uranium in urine has recently been described[174]. This method is based upon thermal ionisation mass spectrometry, and remains relatively expensive at this time.

3.6.4 Urine specimen collection considerations

A decision must be made as to whether a random urine sample will suffice, or whether 24-hour collection is necessary. Single voided urine samples are susceptible to fluctuations in urine concentration due to position, fluid intake, etc. On the other hand, reliable 24-hour collections are notoriously difficult to collect, even in a hospital setting. They are even more difficult in a working population, where the collection is unsupervised for much of the study period[54]. Some constituents are unstable in urine, and for this reason a shorter collection period would be preferable. Creatinine is frequently used as an endogenous substance to correct for urine flow rate in the spot sample[175]. It has been shown that the ratio of total urine protein to creatinine in a spot sample corresponds closely to the values measured from a 24-hour collection, and it has been claimed that the protein/creatinine ratio on a spot sample is potentially more accurate than on a 24-

hour collection because it eliminates error due to incomplete collection[176]. This has been confirmed by others who have studied the correlation between the ratios for urinary protein/creatinine (mg/g) in untimed urines with 24-hour samples from the same subjects[177]. A close correlation was found for all rates of protein excretion ranging from normal (mean 44 mg/day) to nephrotic (maximum 19,300 mg/day). It was proposed that a ratio of <100 mg/g in spot urine samples obtained in the absence of exercise, fever, or other evidence of urinay tract disease, may be used as a criterion of normal kidney function.

3.6.5 Sample preservation

In field studies, hours or days may be required to transport the samples to the laboratory. Some analytes are subject to rapid degradation. In practice, the method of preserving samples must be tailored to the substances to be measured and may be complicated if the preferred technique of preserving urine for one analysis interferes with other desired tests[54].

3.7 Diagnostic renal imaging

For completeness, some applications of diagnostic renal imaging have been reviewed. Among the nuclear medicine approaches, a variety of agents are used which can reflect effective renal plasma flow, GFR, obstruction, and the presence of masses. Cellular biochemistry may be assessed non-invasively by nuclear magnetic resonance imaging (NMRI). This technique holds much promise for medicine, but as yet its precise value remains unknown, and it is still very expensive at this time[178]. The ^{13}C-NMR spectral analysis of urine has been recently reported in the detection of biochemical changes associated with experimentally induced kidney dysfunction in the rat[179]. Tris(2,3-dibromopropyl)phosphate (Tris-BP) was administered, and induced proximal tubular necrosis (most severe in the pars recta and pars convoluta). Serial ^{13}C-NMR excretion profiles of metabolites in the urine were shown to correlate well with histopathological and biochemical assessments over the sequence of events in the nephrotoxic process. The role of this technique remains to be fully evaluated in the assessment of renal injury, especially in terms of specificity.

3.8 Markers of proximal tubular dysfunction / injury: summary and recommendations

As shown above, increased urinary excretion of low molecular weight proteins or high molecular weight enzymes has been observed in a variety of situations. The causes can be divided into drugs, metals, circulatory, immunological, as well as rare renal tubular disorders. The kidneys are particularly susceptible to the damaging effects of drugs and metals, which can achieve their highest concentrations in that organ. Parameters such as urinary β_2-m excretion will detect a 1% change in tubular function, whereas our measures of GFR (plasma creatinine or creatinine

clearance) could miss a 60% reduction in GFR. This gain in sensitivity has the potential to result in a flood of apparently abnormal results; it must be clear that a difference such as increased β_2-m excretion can exist without a detectable disadvantage to the patient[61]. This questions the clinical usefulness of such tests in a non-specific screening mode. In more selective situations such as occupational exposure to nephrotoxins such as cadmium or uranium, longitudinal analysis may be applied and the clinical utility may be improved.

As shown above, there are many tests available which are able to detect early disturbances of renal functional and/or morphological integrity induced by long term exposure to nephrotoxins. Some of these tests are still at the research stage, and much epidemiological work is required to determine their sensitivity and specificity for renal effects[57]. The assessment of the health significance of such early renal changes constitutes another important issue. Are these changes predictive or not of the occurrence of renal disease if exposure to the agent responsible for their appearance is allowed to continue? Experimental and epidemiological studies are urgently needed to assess the prognostic value of these indicators in the occupational setting[57].

There are many factors to be considered when recommending one or more of these markers to screen for occupational uranium nephrotoxicity. Sensitivity and specificity for tubular injury, and ease of analysis have been documented here in the light of available literature. No single marker seems ideal in all respects, and there seems merit in recommending use of a small panel of tests, at least for a pilot study, to determine which ones are the most efficient and practicable for routine application.

In general, the use of urinary enzyme determinations seems to be complicated by a large number of interfering factors, but use of a well documented enzyme such as NAG has the potential to demonstrate tubular cellular injury which may or may not also be accompanied by demonstrable dysfunction. It is therefore recommended that NAG be included for evaluation in initial studies.

At this time, the use of β_2-m and RBP have received the most attention in tubular proteinuria and can be particularly recommended for trial and comparison in uranium exposure. Care must be taken to minimise degradation of β_2-m, and the most practicable approach would be to use a double void collection as outlined above, adding an aliquot of the specimen to a pre-buffered sample container. Newer analytical techniques for the determination of RBP will probably lead to the wider availability of this test at reasonable cost, and may result in reduced demand for use of β_2-m. In a pilot assessment of these markers in the context of uranium exposure, it may also be useful to consider evaluation of α_1-m and ADBP; these markers have received somewhat less attention than β_2-m or RBP, but may offer advantages in terms of stability over β_2-m.

The excretion of all of these urinary analytes should be calculated and expressed in terms of fractional excretion (versus creatinine excretion).

4. RECOMMENDATIONS FOR FURTHER STUDIES AND OCCUPATIONAL MONITORING

Task 6. Taking into account the various chemical forms of uranium found in the nuclear fuel cycle, indicate:

i) what experiments, if any, could be conducted to quantify more accurately the risk;

ii) what tests could be conducted on uranium workers, and on what scale these tests should be conducted, to assess the current state of kidney function in the uranium worker; and

iii) what data should be collected to serve as a basis of future epidemiology studies.

4.1 Experiments to more accurately quantify the risk

The acute nephrotoxicity of several uranium compounds has probably been studied in more detail than any other group of chemical toxins. Questions remain, however, regarding the potential for reversibility of the early renal lesion following subchronic or chronic exposures. Following experimentally-induced acute uranium nephrotoxicity, surviving animals show lesions which never seem to completely regress. Many biochemical parameters return to normal, but the persistent morphological changes in the kidney raise the possibility that renal functional capacity may be permanently impaired. These experimental observations must be weighed against the few reported human acute exposures, where in some cases adverse effects on renal function have been minimal or even undetectable. The critical renal tissue uranium concentration has not been defined for humans, and extrapolation from animal studies is complicated by large inter-species differences in uptake and renal sensitivity. There is no evidence to suggest that the health effects of some chemical forms of uranium found in the nuclear fuel cycle can be ignored, but there is a clear theoretical basis for the assumption that the more sparingly soluble compounds may be of lesser significance. More information is required about the following:

i) mobilisation of various uranium compounds from human lung, and specifically, correlations between inhalational exposure and renal tissue uranium concentration

ii) better estimates of the critical renal threshold in humans (ie the lowest renal tissue uranium concentration at which adverse effects can be observed)

iii) assessment of reversibility of the early renal lesion following chronic exposure

For obvious reasons, it is difficult to design human studies to to address these information gaps. Experiments should be designed using species which are believed to correlate most closely with humans at the tissue level. Baboons are probably the species of choice, but smaller mammals are much more practicable. For intravenously administered uranium, the rat is believed to correspond most closely to humans. The rabbit has a similar gastrointestinal absorption of uranium to humans and may be preferable for orally administered studies. Inhalational studies of uranium compounds have been carried out in the past, but these could be repeated using sensitive markers of renal tubular function as described above to monitor renal effects. Serial sacrifice would provide tissue to derive dose-response data which could then be correlated with the inhalational exposure. Reversibility of the early renal lesion following chronic exposure is a very important issue which deserves close attention. Subacute (24 day) exposure to uranyl fluoride in the rat resulting in peak renal tissue uranium concentrations of up to 5.6 μg/g has been shown to be completely reversible within 35 days following the last exposure[25]. Reversibility studies following chronic exposure have not been reported; these could be designed using a rat or rabbit model, also applying markers of tubular function to monitor progression or recovery of the functional or morphological lesion.

4.2 Current state of kidney function in uranium workers

4.2.1 Opportunity for dose-effect observations

In recent decades, stringent air monitoring in nuclear fuel production facilities has provided accurate information regarding local inhalational exposures. At the same time, changes in operating procedure have generally reduced inhalational exposures from previous levels. More detailed employment records may now enable researchers to more accurately estimate such exposures on an individual basis, and seek correlations with measurements of renal function. If sensitive indicators of renal tubular dysfunction were used in this assessment, this may provide an opportunity to make dose-effect observations which would be otherwise difficult to extrapolate from animal data.

4.2.2 Epidemiological versus clinical objectives?

In designing an occupational study, it is important to distinguish between epidemiological and clinical objectives[54]. Tests used epidemiologically to determine whether there are measurable and/or statistically significant differences between an exposed group of workers and a comparable reference group may not in themselves be diagnostic for an individual worker. Differences between groups can occur without any worker being clearly diseased. Establishing a diagnosis for an individual worker requires additional clinical follow-up. For clinical purposes, the screening tests

are primarily useful as a first step to identify those workers for whom more complete diagnostic evaluation is indicated.

4.2.3 Suggested occupational study design

For a pilot study, a single nuclear fuel production facility could be chosen. Preferably, this facility should be reasonably close to a non-nuclear industrial facility (without exposure to known nephrotoxins) which could provide a control population. The cooperation of the occupational health personnel and workers would be sought, and appropriate consent forms developed. Workers from the uranium facility would be recruited into the study if they had no known confounding urinary tract disease (eg diabetic nephropathy), and if a good exposure history were available. A detailed medical, smoking and drug history would be taken. The study group should be 50-80 in number, and each uranium worker would be age- and sex-matched with one or more workers from the control facility.

Fasting blood would be drawn for the following:

i) chemical profile, including glucose, electrolytes, urea, creatinine, total protein and albumin

ii) β_2-m and RBP, $\pm$ α_1-m

A double void urine sample would be collected, submitted for on-site urinalysis, osmolality and microscopy, and aliquotted for the following determinations:

i) creatinine, total protein

ii) β_2-m, RBP, NAG $\pm$ RTE, α_1-m, ADBP (all expressed as fractional excretion)

iii) uranium

In addition, aliquots of serum and urine would be frozen for possible further investigation. This testing profile must be standardised for collection time, and should be repeated 2 or 3 times for each subject to assess inter-individual variation. Glomerular filtration rate would be calculated for each individual. Serum β_2-m and RBP would be required to confirm that excess excretion, if detected, reflects tubular proteinuria rather than serum levels in excess of the renal threshold. The renal function of each worker would be compared with his controls. The study group could be stratified on the basis of cumulative exposure, and compared with the control group.

Taking into account the experience gained from the pilot study, a subsequent definitive study would then be designed and carried out, on a larger number of uranium workers. If significant alterations in renal function were substantiated among the uranium workers, then recommendations could be made regarding a suitable protocol for long-term monitoring of occupational health. Ideally, this would be based upon use of the most sensitive and specific indicator of such dysfunction with a low analytical cost.

4.3 Future epidemiological studies

4.3.1 Long-term monitoring

Further to the above, it is difficult to provide more specific recommendations regarding the direction of future studies. In fact, studies such as those outlined above are needed to better define the most useful indicators of renal function for long-term monitoring, and should provide the best direction. New observations will undoubtedly spawn new questions: for example, what is the significance of an isolated β_2-microglobulinuria? Should such individuals be removed from further exposures? Or is this finding analagous to the transient serum elevation in liver transaminases following moderate alcohol consumption? (which in the vast majority of cases does not imply eventual hepatic failure). Hopefully, future animal reversibility studies will shed light on this before it becomes a practical issue.

4.3.2 Accidental exposures

In accidental exposures reported to date, renal assessment has usually been limited to gross tests of renal function such as serum creatinine, urea and urinary protein, and urinary uranium as an index of exposure As shown above, these are very insensitive tests of renal injury or impaired function. It would be advisable to design an expanded test protocol for use in accidental exposures, incorporating a panel of sensitive tubular function tests such as those detailed above. Such a protocol could be repeated at intervals (eg 6, 12, 24 hours, and then daily until results are available for interpretation). Data such as this could be very useful in demonstrating biochemical reversibility of an early renal lesion in humans.

4.3.3 Uranium registry

The United States Uranium Registry has a tissue program to enroll workers exposed to uranium in a program of post-mortem tissue study[180]. The objectives of this program are to:

a) determine the distribution and levels of uranium in the tissues of occupationally exposed workers

b) compare bioassay measurements of exposed individuals with the results of analyses of tissues obtained at autopsy

c) seek evidence of histopathologic changes related to any uranium deposition that may be present

d) conduct analyses of whole bodies, when available, to obtain more precise data on the uranium burdens, especially in parts of the body that are not usually accessible for direct sampling

Consideration should be given to the institution of such a program in Canada. If accurate exposure histories are available for workers subsequently examined by such a program, this has the potential to provide much needed information about the tissue distribution of uranium, and in particular correlations between the renal tissue concentration and morphological findings in humans.

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APPENDIX

CONTACT LIST OF RESEARCHERS CURRENTLY INVOLVED IN STUDIES OF URANIUM NEPHROTOXICITY AND RELATED AREAS

Task 3 Identify researchers and laboratories currently involved in studying the effects of uranium uptake on kidney tissues. Indicate the relevance of their work to this project.

In completion of this task, it was recognised that a number of researchers who have published in areas other than uranium nephrotoxicity have considerable experience in significantly related fields, such as nephrotoxicity due to cadmium, or assessment of various markers of renal tubular injury. The contact list has therefore been expanded considerably, with a brief notation to indicate the relevant areas of investigation pursued by each individual. A letter was sent to each person listed, outlining the purpose of this report, and inviting each researcher to respond regarding any recent developments or work in progress which might be included here. Where such responses have been received, these have been editorialised and included below.

A.1 Uranium nephrotoxicity

Bulger, Ruth Ellen
Graduate School of Biomedical Sciences
The University of Texas Health Science Center at Houston
Houston, Texas
U.S.A. 77030
Has written a review of experimental renal damage caused by heavy metals, including uranium

Damon, Edward G
The Inhalation Toxicology Research Institute
Lovelace Biomedical and Environmental Resarch Institute
Albuquerque, NM
U.S.A. 87185
Has described the effect of acclimation to caging on the nephrotoxic response in rat (ammonium diuranate). Co-workers Frank Eidson and Bruce Boecker are members of an NRCP committee addressing the area of metabolism, dosimetry and bioassay for various forms of uranium

Diamond, Gary L
Syracuse Research Corp
Merrill Lane
Syracuse, NY
U.S.A. 13210 (315) 425 5100
Has conducted several studies on nephrotoxicity of uranyl fluoride in the rat, including reversibility studies

Domingo, JL
Laboratory of Toxicology and Biochemistry
Faculty of Medicine
University of Barcelona
C/San Llorenc 21
43201-Reus
Spain (977) 31 89 22
Recent report on acute toxicity studies of uranyl acetate in rats and mice. Currently involved in treatment of experimental acute uranium poisoning by various chelating agents, and reproductive studies in uranium-exposed mice

Gilman, AP
Acting Head
Priority Substances Section
Health Protection Building; B-64
Tunney's Pasture, Ottawa
K1A 0L2
Has conducted subchronic uranyl nitrate nephrotoxicity studies in the rat and rabbit, and preliminary studies to assess the reversibility of the early renal lesion

Haley, Diane P
Department of Anatomy
The University of Texas Health Science Center
San Antonio, Texas
U.S.A.
Has described the morphologic changes in uranyl nitrate-induced acute renal failure in the rat (saline drinking versus water drinking)

Harvey, RB
Dr. R.B. Harvey
Department of Veterinary Public Health
Texas A & M University
College Station
Texas
U.S.A. 77843
Has confirmed impaired renal function (following uranyl nitrate) in a chick model

Hishida, Akira
Dr. Akira Hishida
The First Department of Medicine
Hamamatsu University School of Medicine
Hamamatsu
Japan
Reported on effect of saline loading on uranium-induced acute renal failure in the rat

Roth, D
Department of Medicine and Pathology
University of Miami and VA Medical Center
Miami, Florida
U.S.A.
Has demonstrated amelioration of nephrotoxicity in rat by presence of diabetes

Roxe, David M
Section of Nephrology-Hypertension
Northwestern University Medical School
Suite 1230
251 E. Chicago Avenue
Chicago, Illinois
U.S.A. 60611 (312) 908 2729
Has reviewed types of toxic interstitial nephropathy (including uranium)

Stein, Jay H
Department of Medicine
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas
U.S.A. 78284
Review of experimental models of nephrotoxic acute renal failure (including uranyl nitrate)

Zalups, Rudolfs K
Division of Basic Medical Sciences
Mercer University School of Medicine
1550 College St
Macon, GA
U.S.A. 31201-1554 (912) 744 2559
Has studied acute nephrotoxicity of uranyl fluoride in uninephrectomised rats. Currently engaged in studies to examine the renal tubular transport and handling of heavy metals, and investigating efficiency of rescue chelation therapy following acute mecuric chloride exposure

A.2 Uranium metabolism and distribution

Adams, N
National Radiological Protection Board
Harwell, Didcot
Oxon, England
Several publications on kidney and bone retention functions in humans

Bentley, KW
Australian Atomic Energy Commission
Lucas Heights Research Laboratories
Private Mail Bag
Sutherland, NSW 2232
Australia
Assessed hair uranium as indicator of body burden in mice

Hursh, JB
Department of Radiation Biology and Biophysics
University of Rochester
School of Medicine and Dentistry
Rochester, New York
U.S.A. 14620
Several publications re: uranium metabolism in man

Leach, Leonard J
Department of Radiation Biology and Biophysics
School of Medicine and Dentistry
University of Rochester
Rochester, New York
U.S.A. 14642
Chronic inhalational studies with uranium dioxide dust (monkey, dog and rat)

Morrow, Paul E
Department of Radiation Biology & Biophysics
University of Rochester Medical Center
Rochester, New York
U.S.A. 14642 (716) 275 3861
Published major review of biokinetics and toxicology of uranium, and inhalational studies

Rey, BM de
Radiobiology Department
National Atomic Energy Commission
Avenida del Libertador 8250
Buenos Aires, Argentina
Has studied the deposition and toxicity of subcutaneous uranium dioxide in rats

Stradling, GN
National Radiological Protection Board
Chilton, Didcot
Oxon , OX11 ORQ
UK
Several studies on metabolism of uranium dioxide and trioxide after deposition in rat lung

Wrenn, McDonald E
Radiobiology Division
University of Utah
Salt Lake City, Utah
U.S.A. 84112
Metabolism of ingested uranium

A.3 Epidemiological studies of uranium exposure

Dupree, Elizabeth A
Dr. E.A. Dupree
Center for Epidemiologic Research
Oak Ridge Associated Universities
P.O. Box 117
Oak Ridge, TN
U.S.A. 37831 - 0117
Mortality study among workers at a uranium processing facility

Thun, Michael J
National Institute For Occupational Safety & Health
R-16, 4676 Columbia Parkway
Cincinnati, OH
U.S.A. 45226
Several publications on occupationally-induced renal disease; has reported on renal toxicity in uranium mill workers

Waxweiler, Richard J
National Institute For Occupational Safety & Health
Division of Surveillance, Hazard Evaluations and Field Studies
Industrywide Studies Branch
Robert A. Taft Laboratory
4676 Columbia Parkway
Cincinnati, Ohio
U.S.A. 45226
Occupational studies of uranium mill workers and mortality review

A.4 Indices of tubular nephrotoxicity

Bernard, Alfred M
Industrial Toxicology and Occupational Medicine Unit
University of Louvain
1200 Brussels
Belgium
Many publications re: retinol binding protein as a marker of renal tubular dysfunction

Elinder, Carl-Gustaf
Department of Occupational Medicine
National Board of Occupational Safety and Health
S-171 84 Solna
Sweden
Has reported β_2-microglobulinuria in studies of cadmium-exposed workers

Lauwerys, Robert R
Industrial Toxicology and Occupational Medicine Unit
Clos Chapelle-aux-Champs 30.54
1200 Brussels
Belgium
Many publications re: occupational exposure to cadmium and useful screening tests

Ormos G
National Institute of Occupational Health
Budapest
H-1450
P.O. Box 22
Hungary
Use of urinary β_2-microglobulin and retinol binding protein in cadmium-exposed workers

Plummer, David T
Department of Biochemistry
King's College
Campden Hill
London, W8 7AM
England
Has reviewed the use of urinary enzymes in the assessment of renal injury

Prescott, LF
University Department of Clinical Pharmacology
Royal Infirmary
Edinburgh EH3 9YW
UK (031) 229 2477 local 3328
Published a general review on the assessment of nephrotoxicity

Smith Thomas J
Harvard School of Public Health
Boston, Msssachusetts
U.S.A.
Reported on tubular proteinuria in the context of chronic cadmium exposure

Smith NJ
Occupational Medicine and Hygiene Laboratory
Health and Safety Executive
London NW2 6LN
UK (01) 450 8911 local 296
Published on occupational cadmium exposure and tubular proteinuria

Topping, Michael D.
Occupational Medicine and Hygiene Laboratory
Health and Safety Executive
403 Edgware Road
London NW2 6LN
UK (01) 450 8911 local 229
Has reported an EIA technique for measurement of retinol binding protein

Wibell, Lars
Department of Internal Medicine
University Hospital
Uppsala, Sweden
Review of tubular proteinuria. Special expertise with β_2-microglobulin.

Zager, Richard A
Department of Medicine
Ohio State University Hospital
410 West 10th Avenue
Columbus, Ohio
U.S.A. 43210
Proteinuria of tubulointerstitial nephritis. Experience with renal tubular epithelial antigen.